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Antibacterial activity of propolins from Taiwanese green propolis

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ABSTRACT

Taiwanese green propolis is a prenylated flavonoid rich honeybee product and propolins isolated from Taiwanese green propolis exert a broad spectrum of biological activities, such as anti-cancer and anti-oxidant. However, the anti-bacterial effects of Taiwanese green propolis or propolins are still poorly understood. In the current study, the antibacterial effects of Taiwanese green propolis and propolins were evaluated. Results show that the maximum dry matter yields of Taiwanese green propolis were observed in the 95% and 99.5% ethanol extracts compared to other extraction methods. Consistently, the highest concentration of propolins C, D, F and G from Taiwanese green propolis was obtained in 95% and 99.5% ethanol extracts. Propolins inhibited the growth of gram-positive bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes* and *Paenibacillus larvae*). The average minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of propolins from ethanol extracts were 20 μg/ml. Among the propolins, propolin C had the highest antibacterial activity. Furthermore, Taiwanese green propolis also showed antibacterial activity against methicillin-resistant *S. aureus* (MRSA). In conclusion, these results demonstrate that Taiwanese green propolis and propolins have significant antibacterial activity, particularly against gram-positive bacterial strains.

1. Introduction

Propolis is a natural and resinous product collected by honeybees (*Apis mellifera*) from buds and leaves of trees and then mixed with beeswax. Propolis is used by bees for the construction, repair and protection of beehives due to its mechanical properties and biological activity. In humans, propolis has been widely used as a folk medicine worldwide from ancient times. It has been demonstrated that propolis from Europe and China contains high levels of flavonoids and phenolic acid esters [1]. In addition, several studies have reported that the major compounds with biological activity of Brazilian propolis are prenylated *p*-coumaric acids and diterpenic acids [2]. Currently, ten prenylated flavanone derivatives, propolins A–J, have been isolated from Taiwanese green propolis and characterized [3–6]. Propolins C, D, F and G

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are the most abundant propolins in Taiwanese green propolis [4,6]. Furthermore, Taiwanese green propolis has been reported to have a broad spectrum of biological activities, including anticancer [5] and antioxidant [4].

Over the past few years, several studies have examined the antibacterial activity of propolis. The antimicrobial activity of propolis may differ depending on its geographic region and the season [7,8]. Previous study identified season as a critical factor for determining the total propolin levels in Taiwanese green propolis [9]. Overall, the propolis collected from different regions showed activity against gram-positive bacteria, but showed limited activity against gram-negative bacteria [8,10–14]. Taiwanese green propolis has been shown to have antimicrobial activity against gram-positive bacteria [15–17].

The active compounds in propolis vary depending on area and season. Brazilian green propolis is rich in prenylated derivatives including coumaric acid and diterpenic acids [18]. The main biologically activity compounds in propolis from European are flavonoid aglycones and phenolic acids [1,19,20]. Flavonoids and esters of phenolic acids contribute to the antimicrobial activity of propolis [8,21]. However, tropical propolis still exhibited similar antibacterial activity, despite these substances being undetectable. These findings indicate that different substance combinations in different types of propolis are essential for the biological activity [11]. Furthermore, a synergistic effect of antibacterial activity was observed between the flavonoids in propolis [22]. However, the effect of propolins isolated from Taiwanese green propolis, including propolins C, D, F and G and any interaction between propolins on antibacterial activity have not been studied.

The purpose of this study is to evaluate the antibacterial activity of propolins from Taiwanese green propolis.

2. Methods

2.1. Preparation of ethanol extracts

Taiwanese green propolis was purchased from a local commercial company. The propolis was originally collected from beehives located at different regions in Taiwan from May to July 2015 using propolis collectors. Propolis from the collectors at each location was gathered every month and kept at −20 °C until processed. The ground propolis (10 g) was extracted with 100 ml of 99.5%, 95%, 80%, 70%, 60% of ethanol, methanol and diethyl ether for extraction by shaking (250 rpm) at 25 °C for 48 h. For water extraction, 10 g of ground propolis was extracted with 100 ml H_2O by shaking (250 rpm) at 50 °C for 48 h. The extracts were then filtered through a Whatman no. 4 filter paper and reconstituted to its original volume (100 ml) with original solvents.

2.2. Analysis of propolins

The extract was concentrated by vacuum evaporation, reconstituted and then filtered by syringe filter with a 0.22 μm membrane. The extracts were then tested for propolin (C, D, F and G, chemical structures shown in Fig. 1) content by high performance liquid chromatography (HPLC). In brief, an Agilent 1200 HPLC system (Santa Clara, CA, USA) fitted with a programmable UV detector was used in this study. A reverse phase RP-18 column (ZORBAX SB-C18, 4.6 × 250 mm; Agilent, USA) protected with a guard was used throughout the experiments. Twenty microliters of the samples were injected into the HPLC column heated to 30 °C. The mobile phase consisted

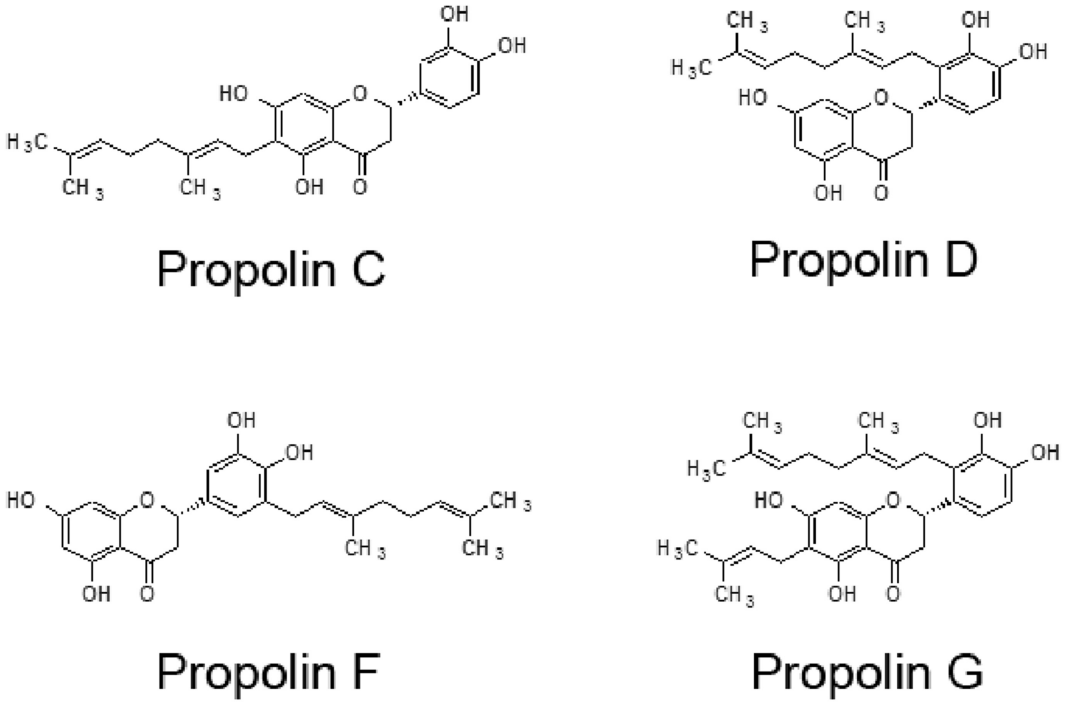

Fig. 1 – Structure of propolins identified in Taiwanese green propolis.

of methanol: water solution (88.8:11.2, v/v). The flow rate was 1.0 ml/min. Propolins were determined at a wavelength of 280 nm by use of a UV detector. The recorder was set to 20 min. Standards were prepared and analyzed a minimum of 3 times. The concentration of propolins was determined based on the slope of the standard curve. All propolin concentrations were determined using linear calibration curves based on the peak area for each propolin. For propolin quantification, linear response was obtained over a range of 1000 to 31.25 μg/ml of propolin standards.

2.3. Test organisms

All bacterial strains were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). After thawing, *Staphylococcus aureus* (BCRC 10780, BCRC 10781 and BCRC 10451) and methicillin-resistant *S. aureus* (MRSA, ATCC 43300) were cultured in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA). *Bacillus subtilis* (BCRC 10255), *Listeria monocytogenes* (BCRC 14845), *Escherichia coli* (BCRC 10675) and *Pseudomonas aeruginosa* (BCRC 10944) were cultured in nutrient broth (NB, Difco Laboratories, Detroit, MI, USA). *Paenibacillus larvae* (BCRC 14187) were cultured in brain heart infusion broth (BHIB, EMD Millipore, Danvers, MA, USA). After two successive transfers of the test organisms in specific culture media, the activated culture was inoculated into specific culture media for further quantification.

2.4. Minimum inhibitory concentration and minimum bactericidal concentration

The antibacterial activity of Taiwanese green propolis and propolins were studied by employing a microdilution method. The Taiwanese green propolis extracts from different solvents were dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) and serially diluted (concentration range from 5.0 to 640.0 μg/ml) for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test. Four propolins (C, D, F and G) were kindly provided by NatureWise Biotech & Medicals Corporation (Taiwan) and serially diluted (concentration range from 0.625 to 640.0 μg/ml) for MIC and MBC test. The 80% of ethanol extracts were used as an indicator of commercial product on antibacterial activities for comparison with propolins. For evaluation of the synergistic effect of propolins on antibacterial activities, each propolin (C, D, F and G) was dissolved in methanol and mixed according to different combinations (propolin C + propolin D group, 2-fold concentration of propolin C + propolin D group, propolin C + propolin D + propolin F group and propolin C + propolin D + propolin F + propolin G group). A concentration range from 0.156 to 640.0 μg/ml was used for the MIC and MBC tests. One hundred microliters of culture broth containing different dilutions was distributed in 96-well plates, as well as a sterility control and a growth control (containing 5% of DMSO). Each test and growth control well was inoculated with 100 μl of a bacterial suspension (10^6 CFU/ml). The MIC value of the extract or propolins was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37 °C. All experiments were performed in triplicate and the microdilution trays were incubated at 36 °C for 48 h. The bacterial growth analyzed by turbidity was detected using optical density (EMax Plus Microplate Reader, Sunnyvale, CA, USA). For the determination of MBC, 10 μL of liquid culture from each well that exhibited no growth were taken and then incubated at 37 °C for 24 h. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC. Positive and negative cultures were also prepared.

2.5. Statistical analysis

All experimental data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC, USA) in a completely randomized design. Duncan's new multiple range test was used to evaluate differences between means. Each broiler formed an experimental unit. *P* values of less than 0.05 were considered statistically significant. For synergy interactions analysis [23], fractional inhibitory concentrations (FIC) were calculated by the formula FIC_A = (MIC A + B/MIC A) or FIC_B = (MIC A + B/MIC B). The FIC index (FICI) for each combination was calculated by the sum of both FIC values, and results were interpreted as follows: FICI $\leq$ 0.5 synergic effect, 0.5 < FICI $\leq$ 4 additive effect and FICI > 4 antagonistic effect.

3. Results

3.1. Antibacterial activity of extracts

After extraction, the maximum dry matter yield from Taiwanese green propolis was observed in the 95% and 99.5% ethanol extracts compared with other extraction methods (Table 1). The dry matter yield was linearly correlated with the concentration of ethanol during extraction. The water extraction exhibited the lowest dry matter yield, indicating that organic solvents were the ideal solution for Taiwanese green propolis extraction. The level of propolins, including C, D, F and G, in the ethanol extracts was further quantified by HPLC using standards (Fig. 2A and B). The maximum yield of propolins (C, D, F and G) from Taiwanese green propolis was also observed in the 95% and 99.5% ethanol extracts compared with other extraction methods (Table 1). Among the propolins, the propolin with the highest concentration in 95% and 99.5% ethanol extract was propolin C. In contrast, propolin F was found in the lowest concentration in the 95% and 99.5% ethanol extracts. The total concentration of propolin C in 95% and 99.5% ethanol extract was approximately 2 times greater than propolin D, 3 times greater than propolin F and 1.5 times greater concentration than propolin G (Table 1). The antibacterial effects of the extracts against *S. aureus* and *E. coli* were examined. Results showed that the average MIC and MBC of organic extraction of propolis for *S. aureus* was 10–20 μg/ml and 20 μg/ml, respectively (Table 2). However, the water extracts were unable to inhibit the growth of *S. aureus* (Table 2). No antibacterial effect on *E. coli* was observed with ethanol, methanol, diethyl ether or water extracts (Table 2). These results demonstrated that the dry matter yield was positively associated with the concentration of propolins. The ethanol, methanol and diethyl ether extracts showed similar antibacterial activity against *S. aureus* and *E. coli*.

Table 1 – Dry matter yield (%) and propolin content (mg/ml) in Taiwanese green propolis extracted using different solvents.[a]

Solvent	Yield (%)	Propolin C (mg/ml)	Propolin D (mg/ml)	Propolin F (mg/ml)	Propolin G (mg/ml)	Propolin C + D + F + G (mg/ml)
99.5% EtOH	66.75 ± 0.5a[b]	14.70 ± 0.28a	7.70 ± 0.15a	4.43 ± 0.10a	9.91 ± 0.29a	36.73 ± 0.80a
95% EtOH	66.25 ± 0.50a	15.06 ± 0.50a	7.80 ± 0.28a	4.47 ± 0.16a	10.21 ± 0.36a	37.55 ± 1.29a
80% EtOH	64.75 ± 0.96b	13.70 ± 0.28b	7.20 ± 0.15bc	4.11 ± 0.09b	9.24 ± 0.20b	34.25 ± 0.71b
70% EtOH	63.25 ± 0.96c	12.63 ± 0.13c	6.95 ± 0.11c	3.76 ± 0.04c	8.20 ± 0.07c	31.53 ± 0.31c
60% EtOH	59.00 ± 1.41d	11.88 ± 0.65d	6.63 ± 0.16d	3.55 ± 0.20d	7.27 ± 0.59d	29.34 ± 1.59d
Methanol	59.75 ± 1.26d	13.31 ± 0.88bc	6.93 ± 0.40cd	3.93 ± 0.27bc	9.18 ± 0.66b	33.34 ± 2.15bc
Diethyl ether	62.75 ± 1.71c	12.07 ± 0.26d	6.37 ± 0.14e	3.74 ± 0.24cd	8.10 ± 0.22c	30.28 ± 0.76d
Water	7.00 ± 0.82e	ND[c]	ND	ND	ND	ND

[a] 10 g propolis was extracted by 100 ml solvent, extracts were finally made up to 100 ml.
[b] Values are mean ± SD. a–e Means within a column with no common letter are significantly different ($P < 0.05$).
[c] Not detected.

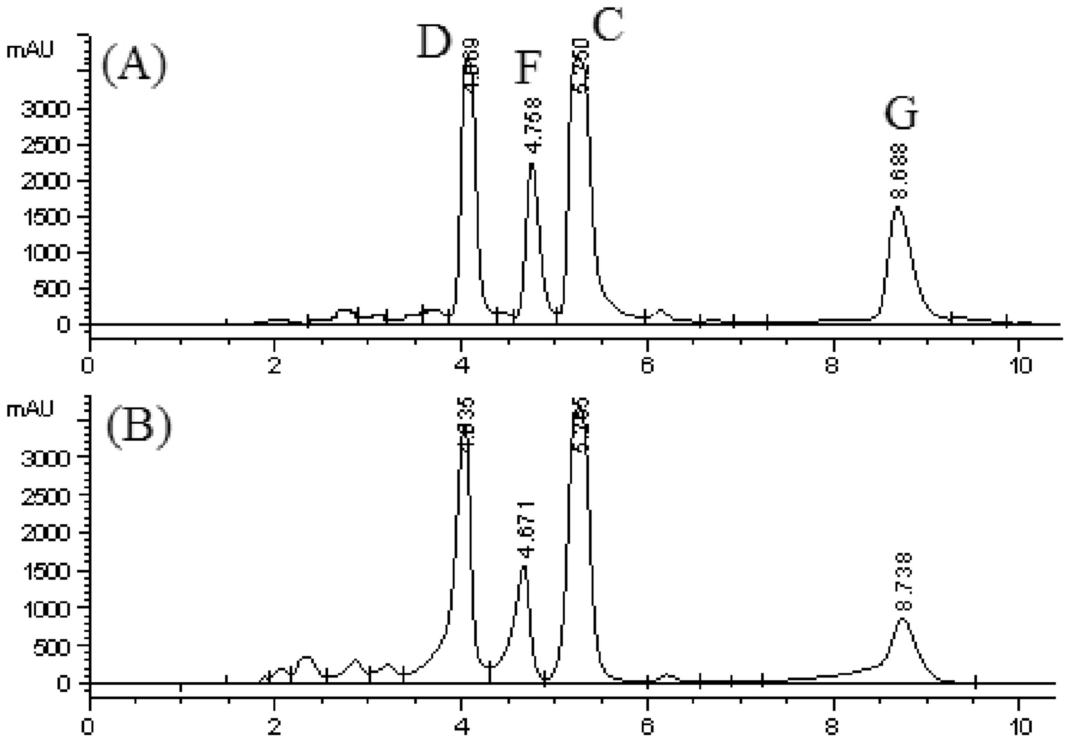

Fig. 2 – Identification of propolins from Taiwanese green propolis. (A) Standards of propolins and (B) measurement of propolins from Taiwanese green propolis by HPLC. Three experiments were carried out, and one representative result is shown.

3.2. *Antibacterial activity of propolins*

Propolin C exhibited the highest antibacterial activity with the lowest MIC against gram-positive strains, including three strains of *S. aureus*, *B. subtilis*, *L. monocytogenes*, and *P. larvae*, with MIC ranging from 1.25 μg/ml to 10 μg/ml (Table 3). The second highest antibacterial activity against gram-positive strains was propolin D (Table 3). Propolin F and G had the similar antibacterial activity against gram-positive strains (Table 3). However, no propolin inhibited the growth of gram-negative strains, including *E. coli* and *P. aeruginosa* (Table 3). Propolin C had strong bactericidal activity with MBCs ranging from 5 to 10 μg/ml against gram-positive strains (Table 4). Similar to propolin C, propolin D also exhibited the lowest MBC against *B. subtilis* and *P. larvae* with MBCs ranging from 5 to 10 μg/ml (Table 4). Overall, propolin D had moderate bactericidal activity with MBC ranging from 5 to 40 μg/ml against *L. monocytogenes* and three strains of *S. aureus* (Table 4). The MBC of propolin G was between 5 and 20 μg/ml against *B. subtilis*, *L. monocytogenes* and *P. larvae*, while MBC of propolin F was between 20 and 40 μg/ml against *B. subtilis*, *L. monocytogenes* and *P. larvae* (Table 4). Propolin F and propolin G had similar bactericidal activity with MBC ranging from 10 to 40 μg/ml against three strains of *S. aureus*. However, none of

Table 2 – MIC and MBC (μg/ml) of different extracts against *S. aureus* and *E. coli*.

Extract	*Staphylococcus aureus*		*Escherichia coli*
	MIC	MBC	MIC
99.5% EtOH	10	20	>640
95% EtOH	10	20	>640
80% EtOH	20	20	>640
70% EtOH	10	20	>640
60% EtOH	20	20	>640
Methanol	10	20	>640
Diethyl ether	20	20	>640
Water	ND	ND	ND

Table 3 – Minimal inhibitory concentration (μg/ml) of propolins and total extracts.

Bacteria strain	MIC of propolin (μg/ml)				
	C	D	F	G	Total extract[a]
B. subtilis	2.5	5	10	10	10
L. monocytogenes	10	20	20	10	40
S. aureus (BCRC 10780)	2.5	10	20	20	10
S. aureus (BCRC 10781)	1.25	10	10	10	10
S. aureus (BCRC 10451)	5	20	20	20	20
P. larvae	2.5	2.5	5	5	5
E. coli	>640	>640	>640	>640	>640
P. aeruginosa	>640	>640	>640	>640	>640

[a] Taiwanese green propolis was extracted with 80% ethanol.

Table 4 – Minimal bactericidal concentration (μg/ml) of propolins and total extracts.

Bacteria strain	MBC of propolin (μg/ml)				
	C	D	F	G	Total extract[a]
B. subtilis	5	5	20	10	10
L. monocytogenes	10	40	40	20	40
S. aureus (BCRC 10780)	5	10	40	40	10
S. aureus (BCRC 10781)	2.5	10	10	10	20
S. aureus (BCRC 10451)	5	20	20	20	40
P. larvae	5	5	10	5	5
E. coli	>640	>640	>640	>640	>640
P. aeruginosa	>640	>640	>640	>640	>640

[a] Taiwanese green propolis was extracted with 80% ethanol.

the propolins had bactericidal activity against gram-negative strains (Table 4). In addition to reference strains, the ethanol extracts were tested for their ability to inhibit the growth of methicillin-resistant *S. aureus* (MRSA). The results showed that the extracts had antibacterial activity with MIC less than 2 μg/ml and MBC of 4 μg/ml against MRSA (Table 5). These

Table 5 – MIC and MBC (μg/ml) of Taiwan green propolis against standard strains.

Bacteria strain	MIC (μg/ml)	MBC (μg/ml)
S. aureus (ATCC 12600)	8	16
MRSA (ATCC 43300)	<2	4
E. coli (ATCC 11775)	>1000	>1000

results suggest that propolin C exhibited the highest antibacterial activity with the lowest MIC and MBC against gram-positive strains compared with other propolins.

3.3. *Combined effect of propolins on antibacterial activity*

The ethanol extracts did not exert as high antibacterial activity as the individual propolins, and propolins represent 50% of the content of the ethanol extracts. Whether there is any interaction among the propolins which affects antibacterial activity remain to be elucidated. The results showed that the highest antibacterial activity with the lowest MIC ranging from 0.3125 to 5 μg/ml against gram-positive strains was observed with two-fold concentration of propolin C plus propolin D compared with propolin C alone (Table 6). The second highest antibacterial activity, with MIC ranging from 0.625 to 5 μg/ml against gram-positive strains was found with propolin C plus propolin D (Table 6). Propolin F in combination with propolin C and propolin D treatment had a negative effect on antibacterial activity compared with propolin C plus propolin D, while propolin G partially improved the antibacterial activity, particularly against *B. subtilis* and *S. aureus* (BCRC 10780). Similar to MIC, the highest bactericidal activity with the lowest MBC ranging from 0.625 to 5 μg/ml against gram-positive strains was observed with a two-fold concentration of propolin C plus propolin D compared with propolin C alone (Table 7). The moderate bactericidal activity with MBC ranging from 1.25 to 10 μg/ml against gram-positive strains was found in the propolin C plus propolin D group (Table 7). Similarly, propolin F in combination with propolin C and propolin D treatment reduced the bactericidal activity compared with propolin C plus propolin D group, whereas propolin G partially alleviated the inhibitory effect caused by propolin F on bactericidal activity, particularly against *B. subtilis*, *S. aureus* (BCRC 10780) and *S. aureus* (BCRC 10451). The combinations of propolins did not cause any bactericidal effects on gram-negative strains (Table 7). Further, propolin C plus propolin D show additive antibacterial activity against *L. monocytogenes*, *Staphylococcus* species and *P. larvae* (Table 8). In contrast, a synergistic antibacterial activity against *B. subtilis* was only observed in propolin C plus propolin D treatment (Table 8). These findings demonstrate that the propolin C has an additive role on antibacterial activity, while propolin F in combination with propolin C and propolin D exerts a negative effect on antibacterial activity.

4. Discussion

In the current study, the ethanol extraction yields maximum dry matter of Taiwanese green propolis. The total propolin levels increased linearly with increasing amounts of ethanol. The ethanol extracts showed antibacterial activity against *S. aureus*. Furthermore, propolin C exhibited the highest antibacterial activity against gram-positive strains compared with other propolins. Propolin C was also observed to play an additive role in exerting antibacterial activity in combination with other propolins.

Table 6 – Combined effect of propolins on MIC against bacteria strains.

Bacteria strain	MIC of propolin mixture (μg/ml)					
	C	C + D	2C + D	C + D + F	C + D + F + G	Total extract[a]
B. subtilis	2.5	0.625	0.3125	2.5	1.25	10
L. monocytogenes	10	5	5	10	10	40
S. aureus (BCRC 10780)	2.5	2.5	1.25	10	5	10
S. aureus (BCRC 10781)	1.25	1.25	0.625	10	10	10
S. aureus (BCRC 10451)	5	2.5	1.25	5	5	20
P. larvae	2.5	1.25	0.625	2.5	2.5	5
E. coli	>640	>640	>640	>640	>640	>640
P. aeruginosa	>640	>640	>640	>640	>640	>640

[a] Taiwanese green propolis was extracted with 80% ethanol.

Table 7 – Combined effect of propolins on MBC against bacteria strains.

Bacteria strain	MBC of propolin mixture (μg/ml)					
	C	C + D	2C + D	C + D + F	C + D + F + G	Total extract[a]
B. subtilis	5	1.25	0.625	5	2.5	10
L. monocytogenes	10	10	5	20	20	40
S. aureus (BCRC 10780)	5	5	2.5	20	10	10
S. aureus (BCRC 10781)	2.5	2.5	1.25	10	10	20
S. aureus (BCRC 10451)	5	5	2.5	10	5	40
P. larvae	5	2.5	2.5	5	5	5
E. coli	>640	>640	>640	>640	>640	>640
P. aeruginosa	>640	>640	>640	>640	>640	>640

[a] Taiwanese green propolis was extracted with 80% ethanol.

Table 8 – Synergy analysis between propolin C and D on MIC and MBC against bacteria strains.

Bacteria strain	MIC of propolin mixture (μg/ml)				MBC of propolin mixture (μg/ml)			
	C	D	C + D	FICI	C	D	C + D	FICI
B. subtilis	2.5	5	0.625	0.375	5	5	1.25	0.5
L. monocytogenes	10	20	5	0.75	10	40	10	1.25
S. aureus (BCRC 10780)	2.5	10	2.5	1.25	5	10	5	1.5
S. aureus (BCRC 10781)	1.25	10	1.25	1.125	2.5	10	2.5	1.25
S. aureus (BCRC 10451)	5	20	2.5	0.625	5	20	5	1.25
P. larvae	2.5	2.5	1.25	1	5	5	2.5	1

Taiwanese green propolis contains prenylated flavanone derivatives and has been shown to have powerful antioxidant and anticancer properties [4,6,15]. Furthermore, ten propolins purified from Taiwanese green propolis also exhibit similar biological activities [3–6]. It has also been reported that flavonoids are the major constituents of propolis and are responsible for its antibacterial activity [8,21,24,25]. Similarly, an inhibitory effect on the growth of bacteria was also observed in Taiwanese green propolis [16,17]. Propolins C, D, F and G are the most abundant propolins in Taiwanese green propolis [4,6]. It has been reported that the structures of propolins C, D, F and G are identical to nymphaeol-A, nymphaeol-B, isonymphaeol-B and nymphaeol-C, respectively [26,27]. To date, only few studies have investigated the effects of propolins on antibacterial activity [28]. Propolins D (nymphaeol-B) and F (isonymphaeol-B) from Egyptian propolis exhibited antibacterial activity against gram-positive (*B. cereus* and *S. aureus*) and gram-negative strains (*Serratia* sp., *Pseudomonos* sp., and *E. coli*). Propolis crude extracts from eastern Australia containing propolin C and G (nymphaeol-C) showed bactericidal effects against *S. aureus* [29]. Here, the propolin C has the highest activity against gram-positive strains than the other propolins. Additionally, previous reports have indicated that propolins from the Solomon Islands, exhibited antibacterial activity against MRSA with MIC values in the range of 64–128 μg/ml [30]. Consistently, Taiwanese green propolis was able to inhibit the growth of MRSA and displayed a MIC of less than 2 μg/ml.

Similar to Taiwanese green propolis, Japanese propolis from Okinawa also contains propolins [31]. Although the level of propolins in Japanese propolis was much lower than Taiwanese green propolis, the propolis from Japan also has antibacterial activity [27]. Furthermore, it has been reported that tropical propolis does not contain propolins but still showed similar antibacterial activity [11]. These results imply that the antimicrobial activity of propolins is complicated and there are different substance combinations in various types of

propolis that are essential for its biological activity. Previously, it has been demonstrated that the level of propolins from Taiwanese green propolis was highly affected by season [9]. Here, current study further identified the interactions between propolins from Taiwanese green propolis. Propolin C had an additive effect on antibacterial activity, which was attenuated in the presence of propolin F. Propolin G partially alleviated the inhibitory effect on the bactericidal activity caused by propolin F. Whether or how propolins may interact with other functional compounds to regulate antibacterial activity remains to be investigated.

Recently, it has been reported that antibacterial compounds found in propolis interacted specifically with the cell wall of bacteria, resulting in cell lysis and eventually bacterial death [32]. This finding implies that the mechanism of bacterial death caused by propolis is structural damage. Since the composition of cell wall between gram-positive and gram-negative strains is totally different. The cell wall of gram-positive strains contains a thick peptidoglycan layer with teichoic acids, while gram-negative cell wall contains a thin peptidoglycan layer that is surrounded by a thick plasma membrane. Currently, the mechanism about how propolis or propolins inhibit the growth of gram-positive strains is still scarce. It is possible that propolins, including C, D, F, and G may interact with the cell wall of gram-positive strains differently due to the structural difference between propolins and all propolins cannot interact with the cell wall of gram-negative strains. Further studies are needed to more fully examine the detailed mechanism.

In conclusion, propolins have diverse antibacterial activity. Propolin C exhibited the highest antibacterial activity with the lowest MIC and MBC against gram-positive strains and had an additive role on antibacterial activity in combination with other propolins.

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